IMMUNOLOGICAL STUDIES IN CHRONIC PNEUMOCOCCUS ENDOCARDITIS.*†

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INTRODUCTION.

IN a previous paper on endocarditis[1] I have tried to show that the continuation of the infection and death are largely due to a process of bacterial immunization against the antibodies of the host rather than to the virulence in the usual sense of the infecting bacteria. It was hoped at the outset of the present study that frequent determinations over a long period, in suitable cases, of the opsonic index, of the antibacterial power of the patient's blood, and of the number of bacteria in the blood in relation to the clinical course and to the injections of dead bacteria and human serum, would throw some additional light on the problems in question.

THE CASES.

Case 362.—M. J., age 52, lumberman, was seen by Dr. Billings, with his physicians, Dr. McKechnie and Dr. J. L. Miller of Chicago, in November, 1908, to whom I am indebted for the opportunity of studying the case.

The patient[2] had been a strong and healthy man all his life, whose business cares were great. He was addicted to the overuse of alcoholics and tobacco. Off and on for several years he had had various muscular ailments and disturbances of digestive organs. During the summer of 1908 he suffered from pyorrhea alveolaris and abscesses of the gums especially about the right upper second bicuspid tooth and also the left upper first molar, for which he received treatment from his dentist. During the late summer of 1908 the gums and teeth were in bad condition, many teeth hung loose. In July, 1908, he noticed lessened strength and endurance, at times he was chilly, and the temperature on a few occasions was found as high as 100. There were transient attacks of pain and some swelling in the various joints, especially of the feet. In the early part of November, 1908, a slight swelling and tenderness in the right great toe led to the diagnosis of gout. From this time onward there was a gradual loss of strength with headache and a temperature ranging from 98 to 99 in the morning to 100 to 101½ in the evening. In the latter part of this month the blood showed 11,000 leukocytes. An agglutination test with typhoid bacilli was said to have given positive results, and the

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[1] *Jour. Infect. Dis.*, 1909, 6, p. 245.

[2] The histories of this and the following case are largely taken from the article by Dr. Billings, "On Chronic Infectious Endocarditis," *Arch. Int. Med.*, 1909, 4, p. 409.

patient was placed on typhoid orders and treated as a typhoid patient for two or three weeks. He then suffered a sudden left hemiplegia and a sudden rise of temperature to 106. The temperature soon dropped to the former range, the hemiplegia improved,

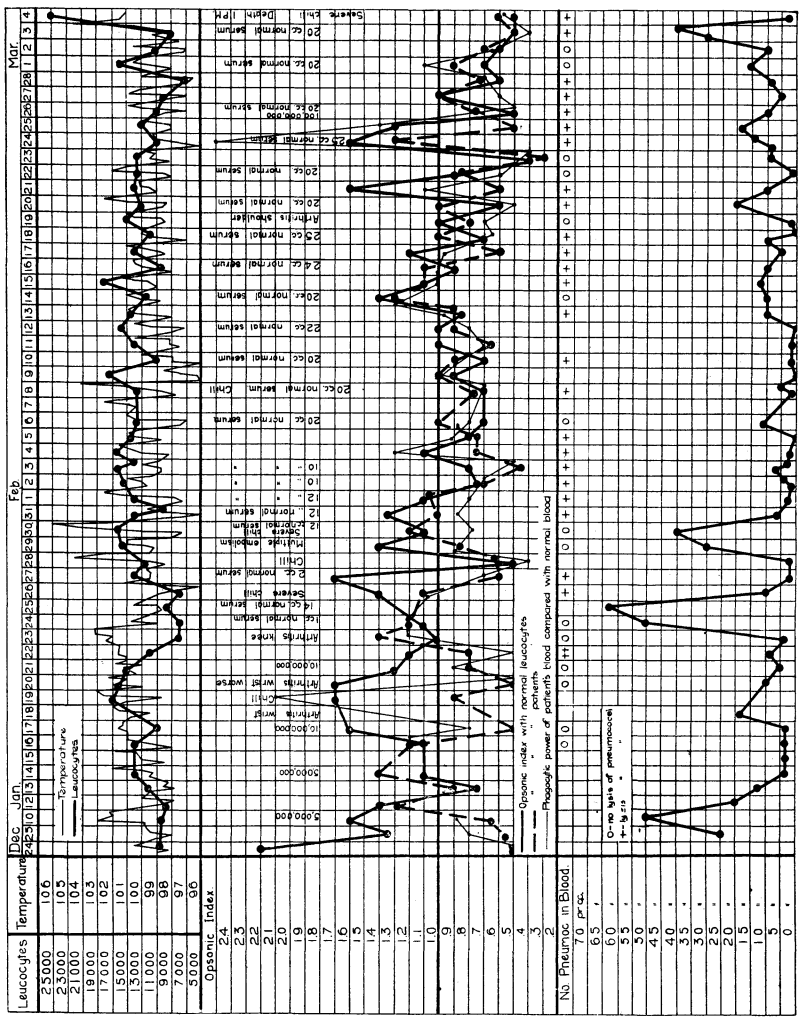

CHART 1 (CASE 362).

and the patient returned to much the same condition as before. The examination of the heart up to this time had given entirely negative results, but from now on a systolic murmur was heard occasionally which later became permanent. Early in December the patient presented a septic type of temperature (see Chart 1), a slight left hemiparesis; he answered questions slowly and took little interest in his surroundings;

there was dilatation of the left heart and now a mitral systolic murmur was detected with transmission to the left as well as accentuation of the second pulmonic tone; lungs normal; liver palpable and somewhat tender; spleen not palpable; a few petechial spots were found in the skin in various parts of the trunk and extremities. The first two blood cultures were sterile, but the third and fourth and the remaining cultures, 53 in all, yielded a modified pneumococcus. On December 13 the Hb. was 85 per cent, the reds 4,000,000, the whites 17,700 with a preponderance of polymorphonuclears. On February 22, 1909, the Hb. was 70 per cent, reds 3,200,000 and the leukocytes 12,000.

Careful chemical analyses of the urine were made by Dr. S. A. Matthews, with, in brief, the following results. A small amount or traces of albumin were constantly present during the last month of the patient's life. The urea at times was increased but averaged 1½ per cent. The urine showed no increase in ammonia or creatinin; on the whole there was a slight diminution in creatinin. From the middle of January till death there appeared periodically variable amounts of a reducing substance which at first failed entirely to respond to carefully controlled fermentation tests in spite of the fact that the gravimetric method showed an average of 1 per cent glucose. Later the amount of glucose ranged between 2.8 to 3.8 per cent and the fermentation test now gave positive results. Tests for acetone, diacetic acid, and B-oxybutyric acid were uniformly negative. No definite relation could be established between the periodic excretion of sugar and the injection of human serum with the resulting reaction (see Chart 1).

Extract of postmortem record (Dr. D. J. Davis): A limited examination only was permitted after the body had been embalmed.

Anatomic diagnosis.—Subacute ulcerative mitral endocarditis; multiple infarcts of the spleen and kidneys; echymosis in the skin; hyperplasia of the spleen; healed and calcified tuberculosis of both pleurae; sclerosis of the aorta; obesity; ossification of the costal cartilages. The spleen soft, enlarged, with several anemic infarcts. The capsule of the kidney strips readily, leaving a smooth surface with two well defined infarcts in each, surrounded by a narrow red zone; the cortex swollen and everywhere studded with small fibrous and calcified nodules from 2 to 3 mm. in diameter. A few occur beneath the pleura in the lung tissue. The heart is large and at the base is a large amount of subpericardial fat. The muscle is firm (probably from the formalin). The pulmonary, tricuspid, and aortic valves are normal and the ascending and transverse aorta show small areas of sclerosis, non-calcified and diffuse. No sclerosis exists about the coronary openings. On both flaps of the mitral valve are large irregular gray masses about 2 cm. across and projecting outward into the lumen. They are firmly adherent to the valve and are covered by dark red blood clots.

Microscopic examination.—There are microscopic miliary abscesses in the liver, pancreas, heart muscle, and spleen. The number of leukocytes, especially polymorphonuclears, is markedly increased in the capillaries of all the organs. There are healed tubercles in the pleura, associated with considerable thickening and slight emphysema and bronchopneumonia. There is considerable fatty change of the liver cells and some round cell infiltration in the connective tissue. There is a marked increase in the cellular elements of the spleen associated with the large number of leukocytes.

Case 408.—A. K., man, married, age 30. I am indebted to Dr. Frank S. Johnson and Dr. Frank Billings for the facts in this case.

During childhood he had acute rheumatic fever with consequential mitral insuffi-

ciency and aortic insufficiency. Compensation occurred and the patient did not suffer until his twentieth year. During his first year at college he attempted rowing, track running, etc., but the result was broken compensation. After a prolonged rest compensatory hypertrophy was restored and he remained in fair condition until November, 1908, when he was operated on for acute appendicitis. He recovered promptly, but several months later there were lessened strength and endurance, a good deal of nervousness and irritability, slight shortness of breath on exertion, with some palpitation of the heart and some fever. Chills followed by fever occurred at irregular intervals. The spleen became palpable, he grew pale, had great dyspnea and palpitation of the heart. A septic type of temperature and a secondary anemia with a low white blood count developed. In May, 1909, the patient was pale, weak; a few petechial spots were found in the skin of the back; the heart was dilated, especially to the left, and the heart action was rapid; a soft blowing diastolic murmur was detected in the aortic area, transmitted down the sternum, and also a loud systolic murmur in the mitral area, transmitted to the left, with an accentuation of the second pulmonic tone. The lungs were clear, the spleen palpable, the liver not enlarged. The urine was practically normal. The blood on May 29 showed 78 per cent Hb., 4,228,000 reds and 8,000 whites. Except for a period early in July when there was a slight increase in Hb. coincident with a decided improvement in the patient's general condition, after the first injection of normal serum, the secondary anemia grew steadily more marked. August 3, 1909, four days before death, the Hb. was 40 per cent and the reds 3,128,000. Two blood cultures during the latter part of May were sterile, while all the subsequent cultures, 37 in all, gave growths of modified pneumococci (see curve in Chart 2). On June 16, 1909, bloody urine was passed; the blood soon disappeared and the urine remained normal until August 5, when blood again appeared, and in addition a large number of hyalin, granular, epithelial, and blood casts. In this case (as in others) the tendency to periodicity of the infection was marked. The occurrence of petechial spots, infarcts of the spleen, kidney, and of several attacks of cerebral embolism, was always associated with prostration, high fever, malaise. A wave of improvement lasting for a variable period occurred after each of these events, the strength, however, being less than before the attacks. This was likewise the case after the injections of dead pneumococci or serum or both combined. For 24 hours after such injection the patient showed signs of intoxication, especially when the bacterial count dropped markedly, but subsequently the patient felt improved. The large doses of pneumococci (200,000,000), given against my judgment, unfortunately exhausted the patient greatly and were not followed by the improvement seen after the smaller doses of cocci alone or serum and cocci together. These large injections seemed to overwhelm the recuperative powers (see Chart 2). The smaller dose, 50,000,000, together with 10 c.c. of normal serum given four days later, had the usual but temporary good effect (July 21 and 22).

A cerebral embolism causing a complete left hemiplegia occurred July 31, four hours after the subcutaneous injection of 10 c.c. of normal serum and 50 million pneumococci. During the night of August 3, four days before death, the patient's respirations grew more rapid, the pulse more bounding, and the temperature higher. This no doubt marked the development of the lobar pneumonia, from which the patient died, August 7, 1909.

Extract from postmortem report.—Anatomical diagnosis: Chronic aortic and mitral and acute ulcerative and vegetative aortic and mural endocarditis; recent infarcts of the spleen and kidneys; acute splenic tumor; lobar pneumonia; acute

fibrinous pleuritis; acute serofibrinous pericarditis; passive congestion and cloudy swelling of the liver; acute nephritis, hypertrophy of the left ventricle (marked); anemia; petechiae; laparotomy scar.

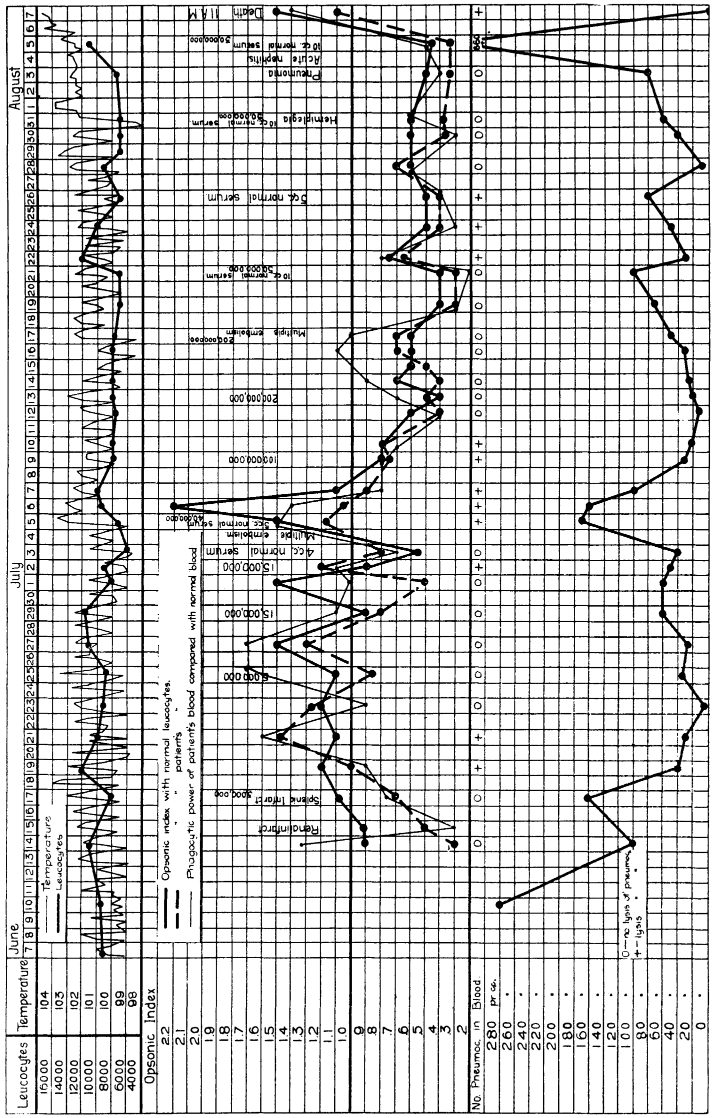

CHART 2 (CASE 408).

The body is that of a well-developed but emaciated young man. The skin shows hemorrhagic areas, varying in size from 2 to 18 mm. in diameter, the largest on the anterior and right side of the abdomen.

The pericardial cavity contains approximately 100 c.c. of a serous fluid in which are small fibrinous masses. The pericardium is smooth and shining. The wall of the left ventricle is much hypertrophied. The endocardium of the right side of the heart is normal. There is a much-thickened calcareous area at the base of the posterior aortic cusp. Several smaller areas are present at the base of the other two cusps. The aortic cusps show no loss of substance and are of normal shape, but are greatly thickened, especially along the free margin, in which there are several calcareous nodes. Growing from the free margins of these cusps are numerous pedunculated vegetations measuring 2–13 mm. in length, all but two springing from the ventricular side. Running down from the posterior cusp there is a large area of ulceration extending over the mural endocardium which covers most of the posterior surface of the posterior leaflet of the mitral valve. This area is 4½ cm. long and 2 cm. in its greatest width. Its base and margins are beset with numerous vegetations, all tending to become pedunculated. Some measure 2 cm. in length. Another smaller but similar area of ulceration is found near the base of the anterior leaflet. It extends downward for a distance of 2 cm. Numerous small areas of ulceration covered with vegetations are found upon the endocardium in the left ventricle directly opposite the large area of ulceration described above. The mitral orifice admits easily three finger-tips, the leaflets are thickened.

The pleural cavities are empty and free from adhesions except for a few over the base of the left lung, which is bound loosely to the diaphragm. The visceral and parietal pleurae show no changes except over the right middle and lower lobe, where it is opaque and rough with a small deposit of fibrin. The whole of the left lung and the upper lobe of the right crepitate throughout. Several depressed feebly crepitant areas are found in the left lower lobe posteriorly. The right lower lobe and nearly all of the middle lobe are dark grayish red in color, firm, and solid. The cut surface is mottled grayish red. There exudes a bloody fluid containing small fibrin plugs. Portions cut from both lobes sink in water.

The spleen contains not less than ten typical anemic infarcts, all hard but friable and surrounded by an area of hyperemia. There are two firm anemic infarcts in the right kidney. The liver was not examined.

Bacteriologic examination.—Smears made from both the right middle and lower lobes show a large number of typical lanceolate encapsulated, gram-positive diplococci, all outside the numerous leukocytes present. Blood agar plates from this material gave a rich pure culture of small colonies surrounded by an abundant greenish zone, markedly different from the zone of green produced by the organism isolated repeatedly from the blood during life. A pure culture of a similar coccus developed in broth. The diplococcus is lanceolate, encapsulated and does not form chains; it is grampositive. It is not susceptible to phagocytosis in normal and in the patient's blood, and when moderate doses were inoculated into a rabbit and guinea-pig it produced a fatal pneumococcemia in 24 hours. No bacteria in peritoneal or pericardial fluid.

The vegetations, after thorough washing in NaCl solution, and on being crushed with a sterile glass rod in this solution, give a moderate number of small gram-positive diplococci in short chains; the leukocytes are fairly well preserved, especially those which do not contain cocci; those which contain diplococci often show a marked disintegration, especially if the ingested organisms show disintegration. A few larger typical lanceolate gram-positive diplococci are also seen, none of which appear in

chains or within leukocytes. Cultures on blood agar plates show a moderate number of typical green-producing colonies, consisting of gram-positive large diplococci, and a much larger number of smaller colonies producing only a trace of green and consisting of small cocci like those isolated so frequently during life. They are freely susceptible to phagocytosis in normal and in the patient's blood.

The heart's blood gave sterile smears and cultures; one typical pneumococcus colony developed on blood agar in approximately 5 c.c. of blood.

Smears from the splenic pulp and infarction show no organisms. Cultures from the pulp result in the isolation of a pure culture of typical pneumococcus, 50 colonies developing upon blood agar plates per drop of pulp.

Case 409.—Salesman, age 26, single, admitted to the service of Dr. Billings at the Presbyterian Hospital, June 4, 1909. Two attacks of acute articular rheumatism 14 and 12 years ago; the heart was involved in both and for a time he was short of breath. Gradually compensation occurred, but he was "short winded" and could not endure the severer athletic sports while at college. Early in April increasing weakness, shortness of breath, and chills followed by fever and sweats came on. The diagnosis of rheumatism was made on account of an attack of pain in the left knee joint and of painful and tender spots with redness and some swelling in the tips of the fingers. Salicylates and a course of injections of mercury previous to entering the hospital failed to relieve the symptoms.

On admission there were marked pallor, dyspnea on slightest exertion, throbbing of vessels of the neck; tonsils of normal size, throat normal; moderate cyanosis, dry hacking cough; regular but quick almost "water-hammer" pulse, heaving precordial impulse. Left border of heart 13 cm. to left of median line; apex diffuse in sixth interspace. Right border 2 cm. to right of sternum. Churning systolic and diastolic murmurs. No edema of hands or feet. Lungs and pleurae free. Occasional petechial hemorrhages in the skin and painful nodes in tips of fingers.

The patient rapidly grew worse, became more and more short of breath and cyanotic; the precordial dulness increased. The day before death the left border and apex impulse were found in the mid-axillary line; edema of the feet, hands, and lungs gradually increased but the pleural cavities remained empty. No demonstrable ascites at any time. The patient died from exhaustion 18 days after admission.

The urine was scant, highly colored, remained free from blood and casts throughout; a trace of albumin was found on two occasions, while two other specimens showed none. The blood showed 4,240,000 reds, 9,600 whites, Hb. 53 per cent, and color index 6. The blood cultures in this case, six in all, each yielded from 30 to 532 colonies of pneumococci per c.c. of blood. The coccus was freely and equally phagocytable by normal and by patient's blood. The destructive power of the patient's blood was far below normal on two occasions within four days of death. The number of pneumococci in the blood at that time was large.

Extract from postmortem report.—Anatomical diagnosis: chronic and acute endocarditis of the mitral and aortic valves; infarcts of spleen and lung; edema of lungs; hypostatic pneumonia of right lower lobe; petechial hemorrhages; passive congestion of liver and kidneys; hypertrophy and dilatation of heart (cor bovinum); cloudy swelling of liver, kidneys, and myocardium; healed tuberculosis of lungs; anemia; acute peribronchial and mesenteric lymphadenitis.

The heart weighs 850 grams. The apex is made up entirely of the left ventricle. There are vegetative growths on both the mitral and aortic valves, grayish red in color,

the cusps being largely destroyed; the remnants, especially of the aortic valve, are fibrous and calcified. The growths spring from the thickened calcareous and fibrous areas of the valves.

Bacteriology.—Smears and cultures from the heart's blood, the vegetations of aortic and mitral valves, the peritoneal fluid, and splenic infarct yield a pure culture of pneumococcus similar to the ones obtained from the blood during life.

This organism is freely susceptible to phagocytosis when grown in broth by normal and patient's blood. It is non-virulent to animals (rabbits and guinea-pigs). When first isolated it forms long chains; grows in the fibrin clot of the blood cultures in large colonies; adheres slightly to the surface of agar and very closely in the colonies so that it is difficult to make even suspensions in salt solution, properties which are soon lost when cultivated on artificial media, when it soon grows like the typical pneumococcus. The organism invariably produces green on blood agar, never hemolysis, ferments inulin slowly, grows slightly in gelatin at 20° C.; acidifies and coagulates milk.

Plate cultures from a small area of consolidation in the lung yield a large number of the pneumococci above described, a few colonies of Strept. pyogenes and Staph. albus. Smears from the vegetations show that they are made up largely of diplococci and short chains and leukocytes held together by a fibrinous network. The appearance, except for the presence of leukocytes, is not unlike the smears made from the large masses which grow at the bottom of broth inoculated with these organisms. Most leukocytes contain no bacteria. Occasionally some are found which contain a large number. The leukocytes thus engaged in phagocytosis show marked disintegration while those which show none are well preserved. No lymphocytes and only few endothelial cells can be found. No eosinophiles.

TECHNIC.

Blood cultures and bacterial counts.—At first the blood cultures in each case were made by drawing from 10 to 20 c.c. of blood from the vein at the bend of the elbow and planting it in liquid media and agar. After a sufficient number of cultures were made to establish the diagnosis and identity of the bacteria, later cultures to determine the number of bacteria were made from the lobe of the ear. The latter method is reliable for estimating the number of bacteria circulating in the peripheral blood in the cases studied, because in 15 simultaneous cultures from the vein at the elbow the same average number of bacteria was obtained. Sterilizing the lobe of the ear as well as possible with green soap and alcohol, and then collecting the blood in a pipette directly from the puncture and not by dropping it into a tube, reduced greatly contamination with skin staphylococci. Over 75 per cent of the plates thus prepared gave no staphylococcus colonies, but pure cultures of the infecting organism.

Phagocytic experiments.—The usual technic was followed. Smears were made at the end of 15 or 20 minutes and also at the end of 1 hour and of 12 to 24 hours. The average number of bacteria taken up per leukocyte was obtained by counting the number in at least 50 leukocytes and frequently in 200 leukocytes. The percentage of leukocytes engaged in phagocytosis corresponded closely with the bacterial counts. The suspensions were made in NaCl solution from agar-grown pneumococci as well as of 24-hour cultures in dextrose broth. The greatest care was exercised to make the results comparable. The normal blood used for the controls was obtained at the same time as the patient's, the washing was done in exactly the same manner with three changes of 50 times the quantity of salt solution. The number of leukocytes in the washed

blood were controlled by actual counts; differential counts were made to establish that not only the total number of whites but that the number of polymorphonuclears were comparable. The suspensions from day to day were made as nearly alike in density as possible. Carbol thionin chiefly was used, altho frequent controls with Giemsa's and Leishman's stains were made.

Pneumococcidal tests.—The mixtures (see Tables 1 and 2) were the same as used to determine phagocytosis, except that the suspensions of pneumococci were diluted 40 to 50 times. Equal quantities of washed blood, serum, and diluted suspension were drawn into the capillary end of glass tubes and thoroughly mixt. A small loop was plated in blood agar immediately and at the end of 24 hours, at which time repeated trials showed conclusive results are best obtained. The loop contained approximately $\frac{1}{20}$ to $\frac{1}{30}$ of the total mixture. Control tests with larger quantities in test tubes gave similar results. The uniformity in the number of colonies which developed in the plates made immediately show the method is a thoroughly reliable one. Sterile plates, however, are not obtained so often as when larger quantities of blood are used in test tubes. This is no doubt due to the larger surface covered at the sides of the pipette in the mixing process where drying usually prevents the complete destruction of the bacteria by the leukocytes. Smears at the end of 18 to 24 hours of both bacteriolytic and phagocytic mixtures were studied as a means of control of the plate method. Here the erythrocytes serve as a means of estimating the number of bacteria in comparable mixtures.

INTRALEUKOCYTIC DESTRUCTION OF PNEUMOCOCCI FROM ENDOCARDITIS.

It has been shown that the pneumococcidal action of normal and pneumonic blood is due to intraleukocytic destruction of pneumococci.[1] The pneumococci used in my experiments were isolated from the blood and sputum of patients with pneumonia and had become non-virulent by artificial cultivation, and hence were freely susceptible to phagocytosis. In order to study whether the destructive action of blood on endocarditis cocci also is due to the same cause, a series of experiments, of which Table 1 is illustrative, were made.

The upper layer of thoroughly washed blood was decanted and used for leukocytic mixtures while the botton layer containing few leukocytes was used for non-leukocytic mixtures. The experiment in Table 1 was made February 25, after 18 injections of normal serum were given intravenously in Case 362. The destructive power at this time was equal to that of normal blood as shown in Experiments 1, 2, 3, and 4, Table 1. The pneumococcidal power here, as for other pneumococci, is proportionate to the number of leukocytes

[1] Rosenow, *Jour. Infect. Dis.*, 1906, 3, p. 683.

present and to the degree of phagocytosis. Destruction is absent where phagocytosis is prevented by taking away the leukocytes; by addition of broth (Experiments 5 and 6); by heating the serum (Experiments 7 and 8); and by using a highly virulent pneumococcus which is insusceptible to phagocytosis (Experiments 11 and 12). Crucial evidence of the effect of phagocytosis on the growth of pneumococci is afforded in Experiments 7, 8, 9, and 10: It has been found repeatedly that the pneumococcus grows equally well in heated and

TABLE 1.

The Destructive Effect of Leukocytes on Endocarditis Pneumococci.

Each Mixture Contains Equal Parts of Washed Blood, Serum, or Broth Pneumococcal Suspension	Phagocytosis (15 Min.)	Colonies on Blood Agar Plates			
		32–34,000 Leukocytes per c.c.		10 Leukocytes per c.c.	
		Immed.	24 Hrs.	Immed.	24 Hrs.
1. Normal blood+normal serum+ pneumococ. 362	1.9	2	200	1	950
2. Normal blood+serum 362+pneumococ. 362	2.3	5	50	2	600
3. Blood 362+normal serum+pneumococ. 362	3.5	5	0	2	1,200
4. Blood 362+serum 362+pneumococ. 362*	2.6	2	8	1	750
5. Normal blood+broth+pneumococ. 362	0.4	0	5,000	10	5,000
6. Blood 362+broth+pneumococ. 362	0.3	0	4,600	2	4,350
7. Normal blood+heated normal serum+pneumococ. 362	0.2	4	1,000		
8. Blood 362+heated serum 362+pneumococ. 362	0.3	25	2,000		
9. Normal blood+normal serum (heated 10 parts, unheated 1 part)+pneumococ. 362	1.5	6	150		
10. Blood 362+serum 362 (heated 10 parts, unheated 1 part)+pneumococ. 362	2.8	5	14		
11. Normal blood+normal serum+pneumococ. 356†	0.3	6	3,200	5	3,750
12. Blood 362+serum 362+pneumococ. 356	0.3	8	4,500	3	5,400

* This experiment was performed when both phagocytosis and bacteriolysis was nearly normal to the homologous strain.

† Pneumococcus 356 highly virulent.

unheated homologous serum, hence the difference obtained in Experiments 7 and 8 where heated normal serum and heated patient's serum are used, and Experiments 9 and 10, in which one part of unheated serum is added to ten parts of heated serum, cannot be due to differences in rapidity of growth of the organism, but must be due to the activation of the serum with resultant increased phagocytosis and intraleukocytic destruction.

In the experiments bearing on Case 408 also, intraleukocytic destruction took place freely and was readily prevented by reducing or preventing phagocytosis by using non-leukocytic mixtures, by using NaCl solution instead of serum, by substituting heated for unheated

serum, by cultivating the cocci in the serum alone without leukocytes, by using highly virulent pneumococci, and by rendering the homologous cocci insusceptible to phagocytosis by previously growing them in fresh normal and patient's serum.

In certain instances (Tables 2 and 3) when the blood from cases of endocarditis appear to lack destructive power it was restored by

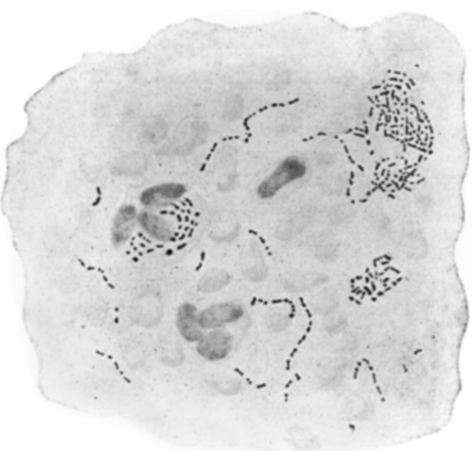

FIG. 1.—Smear made at end of 24 hours of a phagocytic mixture containing equal parts of washed 362 blood, 362 serum, and of pneumococcus 362 suspended in NaCl so u-tion after cultivation on agar. ×1.000.

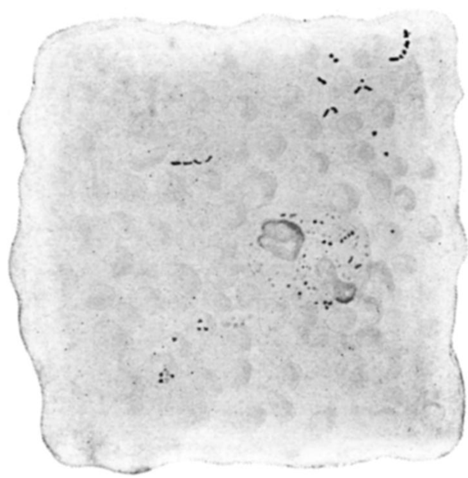

FIG. 2.—The same as (1) except that the mixture contains washed normal blood and normal serum instead of patient's. ×1,000. Note the marked growth, the long chains, the clumps, and the phagocytosis and absence of intraleukocytic destruction of pneumococci in Fig. 1, the leukocytes being well preserved. Fig. 2 shows marked phagocytosis and intraleukocytic destruction of pneumococci with only a few free pneumococci and disintegrating leukocytes.

the addition of varying amounts of normal serum. In mixtures of the same amounts of normal serum and of the patient's serum, but without leukocytes, there would be no destruction; hence the result obtained clearly is not due to the activation of free lysins. This is what would be expected because the organisms grow freely in normal serum and because of the distinct morphologic evidences of intraleukocytic destruction in active mixtures. The study of smears at the end of 18 and 24 hours' incubation (Tables 1 and 2) showed the polymorphonuclear leukocytes to present marked evidence of disintegration in the mixtures in which there was destruction of pneu-

mococci. This disintegration of leukocytes was always most marked where the evidence of intraleukocytic destruction of pneumococci was greatest. The leukocytes in the mixtures where destruction was absent, even though phagocytosis might be marked, and those not engaged in phagocytosis at all, whether in active or inactive mixtures, were always better preserved. Hence the destruction of ingested pneumococci by the leukocytes seems to cost their life.[1]

The activity of the patient's leukocytes as compared with normal leukocytes.—Wright and his followers advanced the view that the source of the leukocytes in opsonic estimations is a matter of indifference, but recent investigations indicate that this view is no longer tenable.[2]

In my own work[3] distinct differences were found in the phagocytic activity of the leukocytes in certain infections as compared with normal, and especially when the phagocytic power of the patient's leukocytes and serum was compared with the phagocytic power of normal leukocytes and serum—opsono-phagocytic index of Glenn and Cox.[4]

Tables 2, 3, and 4 illustrate the results obtained in the series of cases now considered. They show that changes in the leukocytes may play an important part in the phagocytic, and especially the destructive power of the blood.

Table 2 shows that the lack of destruction of pneumococci (Experiments 2 and 4) cannot be due to absence of phagocytosis,[5] because

[1] In this connection the recent work of Werbitzki (*Arch. f. Hyg.*, 1909, 70, p. 270) and Zeissler, (*Mitteil. a. d. Hamburgischen Staatskrankenaustalten*, 1909, 9, p. 167) who studied the destructive power of leukocytes over various pathogenic bacteria, must be mentioned. The former used human serum and leukocytes (obtained from the blood); the latter, rabbits' serum and leukocytes (obtained from the pleural cavity). The number of the leukocytes used in the mixtures was not accurately determined. They concluded that serum and leukocytes have no more destructive power than serum alone.

An analysis of their own tables show that in most instances where the number of bacteria is not too overwhelmingly large the mixtures which contain serum and leukocytes actually show a relatively greater destruction than in those containing serum alone. This is particularly true with respect to staphylococci, streptococci, and pneumococci. Moreover, they take no recognition of the fact that the presence of red blood corpuscles (hemoglobin) in serum makes it a better culture medium especially for pneumococci and streptococci.

In order to secure sterile plates, in a serum-containing mixture, it is necessary to obtain a correct balance between the number of leukocytes and bacteria on the one hand and their susceptibility to phagocytosis on the other. Two factors are involved—growth of bacteria which are free in the serum, and phagocytosis. If the former gets the over hand the leukocytes become packed with bacteria and no evidence of intra-leukocytic destruction can be made out.

[2] T. H. Boughton, *Jour. Infect. Dis.*, 1910, 7, p. 111. Here are given references to the literature.

[3] *Jour. Infect. Dis.*, 1906, 3, p. 683; *ibid.*, 1909, 6, pp. 245 and 296.

[4] *Jour. Path. and Bact.*, 1909, 14, p. 90.

[5] Phagocytosis here is used to designate the mere taking up of the bacteria by leukocytes.

the phagocytosis is alike in the mixtures which show marked destruction of pneumococci and those which show marked growth. The fault seems to be a deficiency of something in the serum which has to do with intraleukocytic destruction quite independently of opsonin.

The experiment described in Table 2 illustrates daily experiments from January 10 to March 4 in Case 262 (Chart 1).

TABLE 2.

PHAGOCYTOSIS AND DESTRUCTION OF PNEUMOCOCCUS 362 BY NORMAL AND BY PATIENT'S (362) LEUKOCYTES IN NORMAL AND PATIENT'S SERUM.

MIXTURES Each mixture contains equal parts of washed blood (20,000 leukocytes per c.mm.), serum, and suspension of pneumococcus 362	PHAGOCYTOSIS (15 MIN.)	SMEARS AFTER 24 HOURS AT 37° C.	COLONIES ON BLOOD AGAR PLATES: Immed.	COLONIES ON BLOOD AGAR PLATES: 24 Hrs.
1. Normal leukocytes and normal serum	1.6	Phagocytosis moderate; decided evidence of digestion of pneumococci; some leukocytes show marked disintegration, others intact	30	14
2. Normal leukocytes and serum 362	1.1	Phagocytosis marked; no intraleukocytic digestion; many diplococci in long chains and clumps; leukocytes well preserved	35	2,500
3. Leukocytes 362 and normal serum	1.2	Phagocytosis slight; marked evidence of intraleukocytic destruction of pneumococci; some leukocytes which show destruction of pneumococci markedly disintegrated; the others stain well; few free pneumococci	28	15
4. Leukocytes 362 and serum 362	1.4	Phagocytosis marked; no intraleukocytic digestion; many diplococci in long chains and bunches; leukocytes well preserved	22	1,550
5. Leukocytes 362, normal serum, and serum 362, equal parts	1.9	Phagocytosis slight; intraleukocytic digestion decided; few diplococci; leukocytes not well preserved	20	40
6. Leukocytes 362 and serum (normal 1 part and serum 362, 6 parts)	1.2	Phagocytosis moderate; marked evidence of intraleukocytic destruction; few free pneumococci; leukocytes showing the greatest destruction of pneumococci are proportionately disintegrated	26	150
7. Leukocytes 362, and serum (normal 1 part and serum 362, 12 parts)	1.6		23	25
8. Leukocytes 362 and serum (normal 1 part and serum 362, 48 parts)	1.7	More growth than in experiments 2 and 5; chains are shorter; more diplococci	47	370

The experiment described in Table 3 illustrates frequent experiments from June 10 to August 7 in Case 408 (Chart 2).

In Table 3 the lack of destruction is confined to the mixture in which the patient's serum and leukocytes were combined (Experiment 4) and appears to be due to diminished phagocytosis. Phagocytosis and intraleukocytic destruction are up to the normal in the mixtures containing the patient's serum and normal leukocytes and the patient's leukocytes and normal serum (Experiments 2 and 3).

On what does the peculiar behavior toward the infecting bacteria of the patient's blood, as illustrated in Tables 1 and 2, and by the curves in Charts 1 and 2, depend? In Case 362 (Table 2) the fault seems to lie with the serum alone, because normal and patient's leukocytes behave alike in the patient's serum. The serum in this instance, while it has practically a normal amount of opsonin, is deficient in something which is necessary to destroy the ingested cocci. Now this lack of destructive power, even tho phagocytosis was up to the normal standard, was always specific for the infecting pneumococcus.

TABLE 3.

Phagocytosis and Destruction of Pneumococcus 408 by Normal and by Patient's (408) Leukocytes in Normal and in Patient's Serum.

Mixtures Each mixture contains equal parts of washed blood (25,000 leukocytes per c.mm.), serum, and suspension of pneumococcus 362	Phagocytosis (20 Min.)	Smears after 24 Hours at 37° C.	Colonies on Blood Agar Plates	
			Immed.	24 Hrs.
1. Normal leukocytes and normal serum	2.58	Phagocytosis marked; digestion of bacteria and leukocytic disintegration; few bacteria, mostly diplococci	2,300	175
2. Normal leukocytes and serum 408	2.2	Same as above, but long chains and clumps	2,150	250
3. Leukocytes 408 and normal serum	2.66	Leukocytes better preserved	2,675	189
4. Leukocytes 408 and serum 408	0.7	Phagocytosis marked; digestion absent; leukocytes well preserved; marked growth; long chains and clumps	2,200	4,300
Leukocytes 408+				
5. Serum (normal serum 1 part, serum 408, 1 part)	2.7	Phagocytosis decided; digestion of bacteria present; leukocytes only fairly well preserved; agglutination; few bacteria	2,000	75
6. Serum (normal serum 1 part, serum 408, 5 parts)	2.4		2,350	125
7. Serum (normal serum 1 part, serum 408, 10 parts)	2.0		1,890	60
8. Serum (normal serum 1 part, serum 408, 50 parts)	2.9		2,750	120

In Table 3, on the other hand, where both phagocytosis and destruction are below normal, it seems to be a fault of the patient's leukocytes when in their own serum, because they are as active as normal leukocytes in normal serum.

Experiments with other strains of pneumococcus, with streptococcus, and with staphylococcus show that in mixtures of homologous serum and leukocytes, low phagocytosis was largely, but not always, peculiar to the infecting pneumococcus.

The study of phagocytosis and intraleukocytic destruction of pneumococci from day to day in these cases shows that the two

processes, while they usually run hand in hand, seem to be independent. Whenever phagocytosis was low in the mixtures containing patient's serum or leukocytes, destruction was correspondingly slight also, but on the other hand when phagocytosis was well up to the normal or above, destruction was usually, but not always, correspondingly marked. Phagocytosis by patient's leukocytes as well as by normal leukocytes might be more marked than in normal blood, yet intraleukocytic destruction in the patient's serum might be entirely absent. The addition of normal serum to the patient's serum in these cases always brought the phagocytosis and destruction up to the normal where either or both were deficient (see Tables 2 and 3), but at no time did it raise the phagocytic or destructive power to a point above that of normal blood.

It was also found that the phagocytic activity of the leukocytes of endocarditis patient 408 varied greatly from day to day, especially when tested in the patient's own serum. In 81 tests the phagocytic activity of the patient's leukocytes with normal serum—cytophagic index of Glenn and Cox—was found greater than that of normal leukocytes with normal serum 51 times, less 28 times. The phagocytic activity of the patient's leukocytes when suspended in the patient's serum—opsono-cytophagic index of Glenn and Cox—on the other hand, was greater than that of normal leukocytes in normal serum only 32 times, and less 47 times—practically the reverse of the first.

The opsonic curves in Charts 1 and 2 show that with few exceptions the days on which the opsonic index of the patient's serum is high or low, the activity of the patient's leukocytes in normal serum is correspondingly high or low; and the dates on which the opsonic power of the patient's serum is high or low, as measured with the patient's leukocytes, the activity of the patient's leukocytes in the patient's serum is correspondingly high or low. The phagocytic activity of the patient's leukocytes in normal serum shows less variation from day to day than when they are suspended in the patient's serum. In the patient's serum (Table 2) there is often a drop in phagocytosis, as if the serum had an inhibitory action on the leukocytes. When leukocytes show a lack of activity in their own serum their activity often is normal or greater than normal in normal serum,

as if the normal serum contained something that activated the patient's leukocytes.

In three experiments the phagocytic as well as the destructive power of the patient's leukocytes was brought up to normal by suspending them (after washing) in normal serum for one and one-half hours, then washing again, and adding cocci previously opsonized (Tables 4 and 5).

If the diminished phagocytosis in the patient's serum was due to lack of opsonin and the serum had no inhibitory action on the leukocytes, then the phagocytic activity ought to be the same as that of normal leukocytes with respect to previously opsonized pneumococci (the cocci being washed after treatment with serum). The fact is that in six tests the activity was found to be less than normal in the presence of serum and in six greater than normal when opsonized ("sensitized") cocci were used. In other words, the patient's serum seems to have had a definite inhibitory action on the leukocytes. The phagocytic activity of the patient's leukocytes in normal serum and toward pneumococci previously sensitized in normal serum corresponded closely on these dates, which again indicates that the patient's serum exerted an inhibitory effect on its leukocytes. Another evidence that the diminution in phagocytosis in mixtures containing homologous serum, leukocytes, and pneumococci frequently observed in these cases, cannot be due to the failure of opsonification, but must be due to an inhibitory action of the serum on the leukocytes, is seen in the fact that pneumococci on suspension in the patient's serum remove its opsonin to the same extent as they do that of normal serum.

The curves (Charts 1 and 2) show that the cytophagic index of the patients was often pronounced during the leukocytosis which followed the occurrence of joint involvement, petechiae, and embolic processes, during the natural course of the disease, as well as after the injection of dead cocci or serum or both; finally, and this is more important, the destructive power of the patient's leukocytes in the patient's serum following these reactions was usually up to the normal or above, while frequently greatly reduced or absent previously.

The relationship between leukocytes, serum, and cocci was analyzed more closely by using cocci that had been treated with

serum, or "sensitized," which means that the cocci were suspended in serum at 37° C. for one and one-half hours and then washed once in salt solution. The cocci used in the experiments given in Tables 4 and 5 were sensitized in exactly the same way and suspended after washing in equal amounts of NaCl solution. The suspensions were rather thin, which explains the small amount of phagocytosis in the mixtures containing pneumococcus 408. This strain had been cultivated on agar for only 48 hours previously and was not as freely taken up as pneumococcus 409, which had been grown for 30 days on blood agar. The suspensions of pneumococci for the study of destruction were made by diluting those for the phagocytic tests 40 times.

Table 4 shows that the destruction of pneumococci is closely dependent on the degree of phagocytosis. The destructive action of the patient's leukocytes (Case 408) is more marked than that of normal leukocytes with respect to homologous cocci sensitized in normal serum and less so in case the cocci are sensitized in the patient's serum. Toward strain 409 the leukocytes of Case 408 haye greater destructive action, but here it makes no difference whether the cocci are sensitized in normal or in the patient's serum.

TABLE 4.

PHAGOCYTOSIS AND INTRALEUKOCYTIC DESTRUCTION OF "SENSITIZED" PNEUMOCOCCUS 408 AND 409 IN THE ABSENCE OF SERUM.

MIXTURES Equal parts of suspension of washed blood (15,000 leukocytes per c.mm.) and suspension of sensitized pneumococci		PHAGOCYTOSIS (20 MIN.)		COLONIES ON BLOOD AGAR PLATE			
				Immediately		24 Hours	
		408	409	408	409	408	409
Cocci sensitized in normal serum +	Normal leukocytes..	0.1	1.38	350	1,200	1,570	2,400
	408 leukocytes	0.2	1.42	320	1,650	750	0
Cocci sensitized in 408 serum +	Normal leukocytes..	0.1	3.1	1,250	625	3,200	1,200
	408 leukocytes......	0.32	2.3	780	700	4,300	0

This experiment was made July 7 when the opsonic power of the patient's serum (in the presence of homologous leukocytes) was well up to the normal. The experiment in Table 5 was made two days later, when the opsonic power of the patient's serum had fallen somewhat. Now the phagocytic activity of the patient's leukocytes is greater than that of normal leukocytes whether the cocci are sensitized

in the unheated and heated normal or patient's serum, but there is now no destructive power on the part of the patient's leukocytes with respect to cocci sensitized either in normal or in patient's serum. In the presence of normal serum, however, the same leukocytes digested cocci as well as normal leukocytes. In other words it seems that during these two days the leukocytes have been so modified that their power to destroy the ingested cocci in the absence of serum is wholly lost. The fault here must be due to changes in the leukocyte and not in the serum alone, because normal leukocytes digest the cocci sensitized both in the patient's and in normal serum.

TABLE 5.

PHAGOCYTOSIS AND INTRALEUKOCYTIC DESTRUCTION OF SENSITIZED PNEUMOCOCCUS 408 IN THE ABSENCE OF SERUM.

MIXTURES Equal parts of suspension of washed blood (14,000 leukocytes per c.mm.), and suspension of sensitized pneumococci		PHAGOCYTOSIS (20 MIN.)	COLONIES ON BLOOD AGAR PLATES	
			Immediately	24 Hours
Cocci sensitized in unheated normal serum +	Normal leukocytes	1.6	500	350
	408 leukocytes	2.6	850	4,500
Cocci sensitized in heated* normal serum +	Normal leukocytes	0.26	450	3,200
	408 leukocytes	0.3	650	4,500
Cocci sensitized in unheated 408 serum +	Normal leukocytes	1.06	540	450
	408 leukocytes	2.02	450	3,500
Cocci sensitized in heated 408 serum +	Normal leukocytes	0.1	375	3,280
	408 leukocytes	0.5	650	4,800

* Heated=60° C. for 1 hour.

In view of the possible objection that the smears at the end of 20 minutes are not an accurate measure of phagocytosis, it should be stated that smears were made of the phagocytic mixtures at the end of one hour and of 18 and 24 hours. The phagocytosis per leukocyte at the end of one hour, while greater, corresponds closely to that at the end of 20 minutes. The evidence of intraleukocytic destruction of pneumococci in the smears at the end of 18 to 24 hours was greater in the mixtures in which the plate method showed the greatest diminution in the number of viable cocci. Experiments like these were made on seven other dates with similar results. In further study of the mode of destruction, washed non-leukocytic blood was used side by side with washed leukocytic blood. Here the growth of cocci previously treated in normal or in patient's serum was equally rapid. Phagocytability for normal leukocytes of agar-grown pneumococci treated in normal serum was always found to

run hand in hand with intraleukocytic destructibility. The patient's cocci treated in the patient's serum, on the other hand, may be taken up freely, but they would not be digested by the patient's leukocytes. In other words, phagocytability and destructibility of the cocci previously treated in the patient's serum, while usually running parallel, do not always do so, as in the mixtures containing serum. The lack of destructive power by the patient's leukocytes was found to be specifically related to the homologous cocci previously treated in the patient's serum.

The phagocytic activity of the patient's leukocytes, determined in this way, was greater than normal leukocytes in all the tests made with the cocci previously treated in normal serum and in all but two instances when they had been previously treated in the patient's serum. The increase in the activity of the leukocytes corresponded to the leukocytosis in the patient.

Pneumococci previously treated in serum and than washed are not only rendered phagocytable, but digestible by normal leukocytes. Other things being equal, the number of leukocytes must be larger than where the serum is not removed in order to obtain sterile plates as would be expected, because pneumococci grow quite as readily in washed blood as in the mixtures where serum is present, but the young cocci, not being opsonized, are not taken up and hence the total growth is more marked. The reason sterile plates were not obtained in Tables 4 and 5 is no doubt due to this cause.

The facts obtained in this way regarding the peculiar relationship between homologous leukocytes, serum, and cocci indicate that the serum may affect the leukocyte so as to render it relatively inactive, even to such an extent that it no longer digests cocci sensitized in normal serum. But that the pneumococcus also is responsible to some degree is shown by the resistance of the infecting strain to destruction, whether in the presence of serum or after sensitization.

PHAGOCYTOSIS AND INTRALEUKOCYTIC DESTRUCTION OF ENDOCARDITIS PNEUMOCOCCI AFTER PROLONGED CULTIVATION IN SERUM.

I have shown that cocci from cases of endocarditis become resistant to phagocytosis when cultivated in normal serum, but remain susceptible to phagocytosis when grown in heated serum. The organ-

isms obtained from the blood during life also resist phagocytosis. The following experiments were made to study the effect of the continuous cultivation of pneumococci from endocarditis in normal serum and in the patient's serum on their susceptibility to phagocytosis and their virulence on the one hand, and on the ease with which they are destroyed within leukocytes on the other.

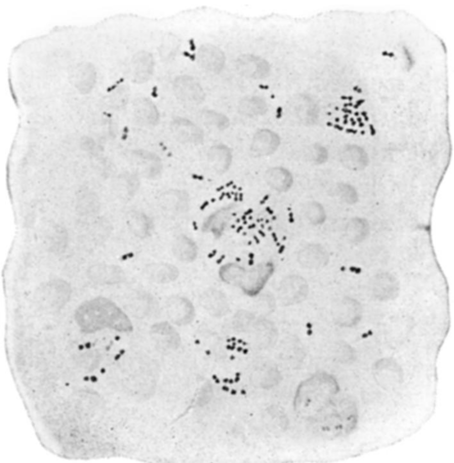

FIG. 3.—Smears made at the end of 24 hours of mixtures of equal parts of washed 408 blood, 408 serum, and suspension of pneumococcus 408 in NaCl. Intraleukocytic destruction of pneumococci and a corresponding leukocytic disintegration. ×1,000.

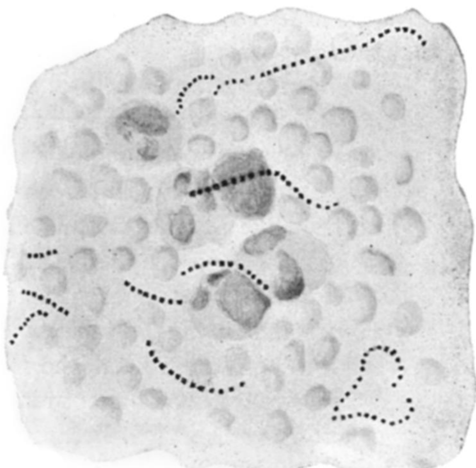

FIG. 4.—The same strain as in Fig. 3 grown in the patient's blood. Note the long chains and the entire absence of phagocytosis. The leukocytes are preserved perfectly. ×1,000.

Beginning July 7, normal and patient's blood (Case 408), obtained on the dates recorded on Chart 2, were used while fresh, as culture media for a strain of the pneumococcus (408), which at no time had been cultivated on artificial media. The amount of phagocytosis and intraleukocytic digestion in these cultures were studied from day to day by making smears from the layer joining corpuscles and serum at the end of 24 and 48 hours. Practically no phagocytosis could be found in the early part of each experiment in either normal or in patient's blood (see Fig. 2); later the cocci became somewhat more susceptible to phagocytosis. This lack of phagocytosis was not due to death of the leukocytes because tests showed that the leukocytes were still active at the end of the experiment. Moreover,

cultivation in washed blood and heated serum showed an enormous apparent phagocytosis at the end of 24 hours. That the acquired resistance to phagocytosis of these bacteria is dependent on substances in the serum which deteriorate rapidly, is indicated also by the fact that phagocytosis was always more marked in the subcultures made after the organisms had been cultivated in the blood without transfer for 48 and especially 72 hours than when cultivated for only 24 hours.

The marked degree of phagocytosis at the end of 24 hours of the organisms cultivated in heated serum to which washed leukocytes are added might be due to phagocytic ingestion of all the cocci or the result of growth within the leukocytes of a relatively small number taken up in the early part of the experiment.

It is also possible that cultivation in serum of the bacteria may render them more resistant to intraleukocytic destruction. A careful study of smears of cultures in normal and in patient's blood at the end of 24 hours on the dates indicated in Chart 2 seems to show that the relatively few organisms which were taken up acquired a resistance to intraleukocytic destruction. See the experiments given in Table 6.

The washed cocci after having been cultivated in serum 408 for 30 days appear highly resistant to phagocytosis in unheated normal and in patient's serum, but apparently freely phagocytable in heated serum. In the second part of the table, in which agar-grown cocci are used, phagocytosis is pronounced in the presence of unheated serum and practically absent in heated serum.

The smears from mixtures containing the washed serum-grown cocci at the end of 24 hours fail to show any evidence of digestion of the relatively few cocci taken up in the presence of unheated serum. The agar-grown cocci, on the other hand, show relatively marked destruction. The cocci which were grown in serum are freely taken up when suspended in heated serum, and show some definite evidence of intraleukocytic destruction by normal leukocytes in normal and in patient's serum, but not by patient's leukocytes in patient's serum. In none of these mixtures, however, was the digestion nearly as marked as in those containing agar-grown cocci in unheated serum. The few agar-grown cocci which were taken up in heated serum show

no evidence of digestion whatsoever. The inability to digest serum-grown cocci was found in the case of those cultivated in normal and as well of those grown in patient's serum. Plates made immediately and at the end of 24 hours showed the growth to be correspondingly

TABLE 6.

Phagocytosis and Intraleukocytic Destruction of Pneumococcus 408 Grown in 408 Serum and on Agar.

Mixtures	Unheated Serum		Serum Heated at 60° C. 1 Hour	
Each mixture contains equal parts of washed blood (18,000 leukocytes per c.mm.), serum, and suspension of pneumococcus 408	Phagocytosis (20 Min.)	Smears after 24 Hours at 37° C.	Phagocytosis (20 Min.)	Smears after 24 Hours at 37° C.
Pneumococcus 408 grown in serum 408 and washed in NaCl solution:				
Normal leukocytes and normal serum	0.20	Decided growth; short chains and diplococci; phagocytosis slight; no digestion	6.40	Marked growth; phagocytosis extremely marked; leukocytes packed with pneumococci; some digestion
Leukocytes 408 and normal serum	0.14		5.30	
Normal leukocytes and 408 serum	0.32	Marked growth; long chains and clumps; little phagocytosis; no digestion	2.30	Marked growth; phagocytosis less than above, but greater than in unheated serum; leukocytes disintegrated; digestion present, but less than where agar-grown cocci are used
Leukocytes 408 and 408 serum	0.08		2.20	Growth marked; phagocytosis slight; no digestion; leukocytes well preserved
Pneumococcus 408 grown on agar:				
Normal leukocytes and normal serum	4.16	Slight growth; few diplococci; phagocytosis slight but digestion marked; leukocytes disintegrated	0.46	Growth marked; diplococci; short chains; phagocytosis decided; no digestion; leukocytes well preserved
Leukocytes 408 and normal serum	3.48		0.46	
Normal leukocytes and 408 serum	1.44	More growth; long chains; phagocytosis decided; digestion present; leukocytes disintegrated	0.36	As above, but longer chains
Leukocytes 408 and 408 serum	1.98	Growth extremely marked; long chains and clumps; phagocytosis marked; digestion slight; leukocytes fairly well preserved	0.66	

greater in the mixtures where no evidences of digestion could be made out even tho phagocytosis is quite considerable. The leukocytes which show phagocytosis but show no digestion and those which contain no pneumococci are well preserved at the end of 24 hours, while those which show evidence of digestion of serum-grown cocci

are disintegrated just as in the mixtures containing agar-grown cocci. The cocci grown in normal serum for 30 days were more susceptible to phagocytosis than those grown in the patient's serum. The agar-grown cocci acquire resistance to phagocytosis in normal serum provided a relatively small number are inoculated; if a large number are introduced they remain freely susceptible to phagocytosis, probably because opsonin is absorbed at the same time as the cocci multiply.

The diminished phagocytosis in Table 7 both by normal and by patient's blood of the organisms previously grown in normal and in patient's blood must be due to relative resistance to phagocytosis; one single subculture in ascites broth, however, rendered the cocci quite freely susceptible. Smears at the end of 24 hours now show distinct evidence of intraleukocytic destruction.

TABLE 7.

RELATIVE RESISTANCE TO PHAGOCYTOSIS OF PNEUMOCOCCI GROWN ON AGAR AND ON SERUM.

PNEUMOCOCCI (No. of pneumococci the same in each mixture.)	PHAGOCYTOSIS (20 MIN.)	
	Washed Normal Blood and Normal Serum	Washed 408 Blood and 408 Serum
Pneumococcus 408 cultivated on agar for 4 weeks and in ascites broth for 24 hours	9.8	3.28
Pneumococcus 408 cultivated in normal blood for 4 weeks and in ascites broth for 12 hours	5.6	1.86
Pneumococcus 408 cultivated in 408 blood for 4 weeks and in ascites broth for 24 hours	3.84	1.12

In the case of pneumococcus 409 similar tests were made by cultivating the patient's strain directly from the blood in the serum obtained after death by transfer every other day for two weeks. At first the cocci resisted phagocytosis completely, but later, as the serum grew old, they gradually became as freely taken up as they were in heated serum in the beginning. Conclusive evidence of intraleukocytic digestion could not be made out.

From observations like those given in Table 6 it is clear that the cocci absorb opsonin freely when grown in fresh normal and patient's serum, because they become susceptible to phagocytosis by washed leukocytes in heated serum, but when thus saturated with opsonin they are insusceptible to phagocytosis in normal and patient's unheated serum. An exact explanation of this peculiar phenomenon cannot be offered. Perhaps the concentration of absorbed opsonin within the

cocci and free opsonin in the serum plays a rôle, because repeated washing of the cocci make them more susceptible to phagocytosis in unheated serum and because those washed only once are taken up in diluted serum much as they are in the heated serum.

The resistance to phagocytosis of serum-grown cocci in unheated serum is somewhat analogous to the unexplained failure of agglutination of certain strains of typhoid bacilli and other bacteria to take place in low dilutions of serum while it occurs freely in high dilutions.

SUMMARY.

A study from day to day of the number of bacteria in the blood, the opsonic power of the serum, the phagocytic and the destructive power of the blood in chronic endocarditis, brings out the interesting facts that the number of bacteria increases and the destructive power of the blood decreases for a variable time previous to the occurrence of embolism and joint infections (see Charts 1 and 2). The opsono-cytophagic index of the patient at this time usually appears to be high. During the reaction after embolism and arthritis, the peculiar behavior of the patient's serum and of the leukocytes disappears, the destructive power of the blood returns, the number of bacteria in the blood shows a corresponding drop, the patient feels better but is weaker than before. Hence the occurrence of embolism and of other intercurrent processes in endocarditis is associated with a definite lowering of the destructive power of the patient's blood.

Phagocytosis and intraleukocytic destruction of endocarditis pneumococci by normal and by patient's leukocytes in normal serum always run hand in hand. Phagocytosis and intraleukocytic destruction by patient's and by normal leukocytes in patient's serum, on the other hand, do not always run parallel. While lowered phagocytosis was always associated with lowered destruction, normal and even increased phagocytosis was frequently associated with complete absence of intraleukocytic destruction and consequent marked growth of pneumococci. Hence phagocytosis and intraleukocytic destruction, while closely related, are independent.

In the instances when no destruction occurs, the patient's serum either is deficient in something necessary to make the cocci digestible or it contains a substance which alters the leukocytes so that they are

unable to destroy the ingested cocci. Certain experiments with sensitized cocci and in absence of serum clear up this point: Normal leukocytes digest the cocci sensitized in the patient's serum quite as readily as those sensitized in normal serum, hence the inability of normal leukocytes to digest the pneumococci in the actual presence of the patient's serum must be due to an effect on the leukocytes by the serum. The patient's leukocytes may be unable to digest the cocci sensitized in patient's serum, even tho more active than normal phagocytically, and yet digest fairly well those sensitized in normal serum; and at another time they may be unable to digest the cocci whether sensitized in normal or patient's serum. This was found to be specific for the infecting strain of pneumococcus. There seems then to be a substance in normal leukocytes, which at times is lacking in the patient's leukocytes and which has to do with intra-leukocytic digestion and is independent of opsonin—a substance which is present in normal serum because patient's leukocytes take it up from normal serum. Normal leukocytes in patient's serum at times digest pneumococci when patient's leukocytes do not. More often, however, normal leukocytes in the patient's serum lose the power to digest the cocci, even tho phagocytosis is normal, while they at the same time digest cocci previously sensitized in the patient's serum and then washed—good evidence that the serum of the patient at times is not only lacking in the substance necessary for digestion, but that it contains something which neutralizes that carried by washed leukocytes and present in small quantities of normal serum. This affords an explanation why smaller quantities than one part of normal to forty parts of patient's serum failed to activate patient's blood *in vitro*. The way normal serum activates the patient's blood then must be by neutralizing this substance and supplying an excess of it which the leukocytes need. In other words, it seems that normal serum has in it a substance which acts on the leukocytes which is independent of opsonin, and which has to do especially with intra-leukocytic digestion. The patient's serum at the time when the destructive power is absent is not only deficient in this substance, but frequently contains an antagonistic body.

A leukocyte count of 10,000 would give 10,000,000 leukocytes per c.c. to take up, let us say, 100 cocci per c.c., which is a fair average of

the number present. This makes 100,000 leukocytes per pneumococcus. The fact that the pneumococci are not destroyed under such circumstances would seem to be good proof that the mechanism by which the cocci protect themselves is a reliable one.

The question whether the cocci actually multiply in the blood in cases like those under consideration has been much discussed. It is usually assumed that they do, but no direct evidence to prove this point has been brought out in the past. The results obtained by cultivating the cocci in defibrinated blood would make one think growth takes place in the blood *in vivo*. Definite evidence has come to light in the study of these cases that in cultures from the blood we are dealing with cocci which have been washed into the circulation from the thrombotic growths on the valves as well as those which have actually multiplied within the blood. The latter differ from the former in that they resist phagocytosis and fail to ferment inulin; the former are freely susceptible and ferment inulin. Similar results were reported in my previous paper. Two factors then seem to be responsible for the continuation of the infection—the focus on the heart valve acting as a feeder, and the multiplication of the cocci within the circulation. The former would seem to resist healing from the protection afforded the cocci by the large thrombotic masses on the valves; the latter by a process of immunization against the antibodies of the host. In acquiring resistance to phagocytosis and what is more important, resistance to intraleukocytic digestion, the cocci also probably produce changes in the serum which alters the leukocytes so that they become less active phagocytically in the patient's serum and less able to digest the infecting cocci.

The therapeutic injection of killed pneumococci in this class of cases holds out little hope in the way of cure. Small doses sometimes seem to produce temporary improvement. Large doses certainly do harm. The injection of normal serum seems to have a decided influence in changing the peculiar conditions of the blood. In cases 362 and 408 the injections were associated with a definite reaction and temporary improvement. The sharp reactions obtained in the beginning from intravenous injections of small doses would seem to indicate that transfusion of blood in these cases might be dangerous.

Horder failed to obtain an immune serum by repeated injections

with cocci from cases of endocarditis of animals. The results of the animal experiments make the outlook for a specific serum therapy gloomy. The more one learns of the mechanism of the infection in these cases the more difficult the discovery of a specific therapy would seem to be.

Finally, in the light of my results there is still another point which should be emphasized. Practically all of the cases of endocarditis give a definite history of a previous endocarditis which has healed, leaving injured and deformed valves. The first attack may have occurred during rheumatism or chorea or following an attack of tonsilitis or from unknown cause. To the rough and at times calcified valves is assigned the greater susceptibility of these individuals to this infection. It is reasonable to suppose that this may aid in the localization of the microorganisms, but it does not, it seems to me, offer any explanation why these cases should progress steadily to a fatal end. The increased susceptibility of rabbits to subsequent inoculations with the cocci from chronic infectious endocarditis suggests that in the case of these patients there also exists an increased susceptibility. The primary attack of endocarditis may alter the mechanism of immunity in man just as the first injection appears to do in rabbits, in such a way that the cocci, tho not virulent in the usual sense, eventually gain the upper end.

CONCLUSIONS.

The following are points that seem to merit specific emphasis:

In chronic pneumococcus endocarditis the serum has no pneumococcidal power by itself.

The phagocytic power of the patient's blood is usually a better index of the actual conditions than the opsonic power of the serum determined with normal leukocytes and even with patient's leukocytes. The destructive power of the patient's blood as compared with that of normal blood corresponds well with the clinical conditions. Hence less reliance should be placed on the results of the opsonin determinations in which no account is taken of the fate of the ingested cocci.

The phagocytic power, as well as the destructive power, of the leukocytes in chronic infectious endocarditis shows greater variation in their own serum than in normal serum. Variations in the opsonic

power of the serum sometimes occur independently of variations in the phagocytic and destructive power of the leukocytes, but the variations on the part of the leukocytes is always dependent on changes in the serum.

While the chief action of serum in phagocytic mixtures is on the bacteria, there is no question, from the evidence at hand, but that the serum also exerts a definite and sometimes a striking influence on the leukocytes. Intraleukocytic destruction is dependent on a substance or property always present in normal serum and in normal leukocytes after treatment with normal serum, but frequently absent in the serum and leukocytes in chronic cases of endocarditis. At times the patient's serum is not only lacking in this substance, but contains another substance which seems to neutralize that in normal serum. This substance is not taken up by the bacteria but the opsonin or some other substance contained in washed sensitized pneumococci is necessary to activate it, because the cocci which have undergone spontaneous phagocytosis fail to show digestion.

In phagocytic mixtures disintegration of leukocytes is directly proportional to the amount of intraleukocytic destruction of pneumococci. Phagocytosis alone, even tho marked, does not seem to injure the leukocytes in any way.

The bacteria isolated from the cases shown appear to be modified pneumococci and prolonged cultivation in normal serum not only renders them largely resistant to phagocytosis, but makes them also resistant to intraleukocytic digestion. Both these properties are promptly lost in artificial media.